Collagen Degradation in Aged/Photodamaged Skin *In Vivo* and After Exposure to Matrix Metalloproteinase-1 *In Vitro*

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Biochemical and ultrastructural approaches were used to assess collagen changes in photodamaged skin. Extensive collagen fragmentation, clumping of the fragmented collagen, and interaction of fibroblasts with the damaged matrix were observed. Similar, though less extensive, collagen damage was also observed in sun-protected skin-individuals aged 80 y or older (naturally aged skin). In comparison, sun-protected skin from young individuals (18–29 y of age) demonstrated little damage. A uniform distribution of collagen fibrils was seen. Interstitial fibroblasts were embedded in the collagen matrix and in close apposition with intact collagen fibrils. In additional studies, three-dimensional lattices of type I collagen were exposed *in vitro* to matrix metalloproteinase-1 (interstitial collagenase), and examined for biochemical and ultrastructural alterations. Under conditions in which enzyme treatment produced fragmentation in 30–40% of the collagen molecules, the lattices demonstrated collagen fragmentation and clumping of the damaged matrix. Recent studies have demonstrated a loss of procollagen production by fibroblasts in contact with collagen fragments *in vitro*. This study demonstrates similar changes in collagen structure *in vivo* in aged and photodamaged skin. We suggest that collagen fragmentation *in vivo* could underlie the loss of collagen synthesis in photodamaged skin and, to a lesser extent perhaps, in aged skin. ***Key words: collagen fibril/dermis/scanning electron microscopy/transmission electron microscopy.***
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Type I collagen is the major structural protein in skin. Collagen destruction, along with damage to the other structural components of the skin (i.e., elastic and reticular fibers) occurring over decades is thought to underlie the characteristic alterations in the appearance of aged skin and the additional changes that result from chronic sun exposure (Smith *et al*, 1962; Schwartz *et al*, 1989, 1993; Maloney *et al*, 1992; Marks, 1992; Bailey, 2001). Mechanisms of collagen destruction in aged or photodamaged skin are not fully understood. Collagen damage is due, at least in part, to degradation by matrix metalloproteinases (MMP) released from epidermal keratinocytes and dermal fibroblasts. MMP levels in skin increase as a function of age (Varani *et al*, 2000). These same degradative enzymes are transiently elevated in direct response to ultraviolet irradiation (Fisher *et al*, 1996, 1997).

Although increased expression of enzymes that degrade collagen is, undoubtedly, responsible for much of the progressive damage in collagen structure/function that occurs in aging and photodamage, decreased synthesis of procollagen also contributes. There is a sustained reduction in collagen synthesis in naturally aged skin as compared with young skin (Varani *et al*, 2000). Superimposed on this is a transient reduction following ultraviolet exposure (Fisher *et al*, 2000) and a further (sustained) reduction in old photodamaged skin as compared with matched sun-protected skin (Griffiths *et al*, 1993; Talwar *et al*, 1995). The difference between young and old individuals is, in part, a reflection of an intrinsic reduction in the capacity of old fibroblasts to synthesize collagen. This is suggested by the finding that fibroblasts from young and old individuals demonstrate the same differences in collagen synthesis *in vitro* as they do *in vivo* (Lovell *et al*, 1987; Varani *et al*, 2000). What accounts for the sustained reduction in procollagen synthesis in photodamaged skin is not fully understood. Intrinsic differences do not appear to underlie differential collagen production in photodamaged skin as compared with matched sun-protected skin because when fibroblasts are removed from the tissue and cultured *in vitro*, differences in collagen-synthetic capacity disappear (Varani *et al*, 2001).

Recently it was demonstrated that when dermal fibroblasts were incubated in contact with type I collagen that had been fragmented *in vitro* by exposure to a mixture of MMP from human skin or to MMP-1 (interstitial collagenase), synthesis of type I procollagen decreased (Varani *et al*, 2001, 2002). This suggested that the presence of damaged collagen may act in some manner to downregulate collagen synthesis by cells that are inherently capable of making collagen. Although these studies clearly demonstrate that type I collagen damaged *in vitro* has a negative effect on procollagen elaboration, how relevant *in vitro*-damaged collagen is to collagen damage in aged/photodamaged skin is not clear. In order to begin addressing this critical question, in this study we have compared damage to reconstituted type I collagen in three-dimensional culture with collagen damage observed *in vivo* in aged and photodamaged skin. Findings from this study demonstrate that damage to type I collagen in three-dimensional *in vitro* culture following MMP-1 treatment (including fragmentation collagen and clumping of the damaged collagen) is similar in ultrastructural appearance to the damage seen *in vivo* in aged skin and (more prominently) in photodamaged skin.

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Abbreviations: MMP, matrix metalloproteinases.

MATERIALS AND METHODS

Skin biopsies Two millimeter, full-thickness biopsies were obtained from forearm skin of 14 individuals ranging in age from 46 to 83 y. All individuals were characterized by the presence of severe photodamage on their forearms. In addition, 2 mm punch biopsies of sun-protected (hip) skin from the same individuals were also obtained. Two millimeter punch biopsies of sun-protected hip skin were obtained from an additional 11 individuals between 18 and 29 y and from 11 individuals aged 80 y or older. Distribution of skin biopsies from individuals in each study group are summarized in **Table I**. The use of human skin in this study was approved by the University of Michigan Institutional Review Board, and all study participants provided written informed consent prior to biopsy. Immediately after biopsy, the tissue samples were frozen in liquid nitrogen and extracted for quantitative assessment of collagen (see below) or fixed in glutaraldehyde for scanning electron microscopy (SEM) or transmission electron microscopy (TEM) as described below.

Quantitative assay for collagen fragmentation in skin Skin biopsies were homogenized in Tris buffer (20 mM; pH 7.3) and centrifuged. The pellet, containing the collagenous extracellular matrix, was resuspended in 150 μl of Tris buffer containing 75 μg of crystalline α-chymotrypsin, and incubated for 8 h at 37°C. The pellet from homogenized skin biopsies incubated in buffer alone served as the control. At the end of the incubation period, the reaction tubes were centrifuged at $10{,}000 \times g$ for 10 min. Supernatants were collected and assayed for hydroxyproline using automated amino acid analysis. Unlike intact fibrillar collagen, which is resistant to α-chymotrypsin hydrolysis, collagen that has been partially degraded by collagenolytic enzymes can be further broken down and the hydrolysis products liberated from tissue extracts by α-chymotrypsin (Bank *et al*, 1997). The amount of hydroxyproline released into the supernatant fluid is therefore a measure of the amount of fragmented collagen initially present in the skin sample. In additional studies with skin biopsies from individuals who were 18–29 y old and aged 80 y or older, we also assessed total hydroxyproline content as a function of total protein to obtain an estimate of collagen content in young and old skin.

MMP-1 Human MMP-1 was obtained from Calbiochem (San Diego, CA). The enzyme was purified from human rheumatoid synovial fibroblasts as the naturally occurring proenzyme form. The MMP-1 preparation appeared as a doublet at 52 and 57 kDa in β-casein zymography and was reactive with rabbit polyclonal anti-MMP-1 antibodies by western blotting. Activation of the proenzyme form was accomplished by exposure to 1 μg of crystalline trypsin for 5 min at 37°C followed by 2 μg of soybean trypsin inhibitor.

Preparation and degradation of polymerized collagen lattices Rat tail collagen (4.7 mg per ml in 1 M HCl) (BD Biosciences, Bedford, MA) was diluted to 1 mg per ml in culture medium consisting of serum-free, Ca^{2+}-supplemented (1.4 mM Ca^{2+}, final concentration) keratinocyte basal medium (MA Bioproducts, Walkersville, MD). The collagen solution was made isotonic by addition of an appropriate amount of 10 × concentrated Hanks' balanced salt solution, and the pH brought to 7.2. The collagen was added to wells of a 24-well plate (0.5 ml per well). The dishes were incubated for 2 h at 37°C, during which time a stiff lattice of polymerized collagen formed (Bell *et al*, 1979).

Table I. Distribution of skin biopsies

	Number of Specimens	
Study group	Hip (Sun-protected)	Forearm (Sun-damaged)
Photodamage (46–83 years)		
Biochemical analysis	9	9
Ultrastructure	5	5
Natural aging (18–29 years)		
Biochemical analysis	8	-
Ultrastructure	3	-
Natural aging (80 + years)		
Biochemical analysis	8	-
Ultrastructure	3	-

Photodamage group: Includes 14 individuals total. Biopsies were taken from sun-protected and photodamaged skin from each individual.

Natural age group: Includes 11 individuals 18–29 years of age and 11 individuals 80 + years of age. Biopsies were taken from sun-protected hip skin only.

Degradation of polymerized collagen was achieved by exposing the collagen lattices to activated MMP-1 for 5 h at 37°C. Collagen lattices exposed to buffer alone served as a control. At the end of the incubation period, the presence of collagen fragments released into the incubation buffer was assessed by resolution in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (8.5% gel) and staining with Brilliant Blue dye. The collagen lattices were then seeded with 8×10^4 human (neonatal foreskin) dermal fibroblasts and incubated for 24 h. At the end of the incubation period, the lattices were fixed in glutaraldehyde and processed for SEM or TEM as described below. In certain experiments, the collagen lattices were treated sequentially after enzyme digestion with 10 mM ethylenediamine tetraacetic acid to inactivate residual MMP activity and 14 mM Ca^{2+} to remove residual ethylenediamine tetraacetic acid. Fibroblast response on the ethylenediamine tetraacetic acid/Ca^{2+}-treated collagen lattices was similar to their behavior on lattices not exposed to ethylenediamine tetraacetic acid/Ca^{2+}, indicating that most of the collagen digestion occurred within the initial 5 h period.

Electron microscopy Skin biopsies and *in vitro* collagen lattices were fixed overnight in 2% glutaraldehyde in 0.1 mM cacodylate buffer (Sigma, St Louis, MO) at pH 7.4. For SEM, postfixation in 1% osmium tetroxide (EM Sciences, Fort Washington, PA) was followed by staining with uranyl acetate and dehydration in graded ethanol. Critical point drying was from absolute ethanol through liquid carbon dioxide. The specimens were then mounted on stubs with double sticky-tape and coated with gold in a dc sputter coater. The specimens were examined using an ISI Super IIIA Scanning Electron Microscope. As SEM provides a three-dimensional image, we were careful to avoid the edges of the tissue. In this way, we were able to avoid focusing on breaks in the collagen molecules resulting from "knife cuts" or mechanical breaks.

For TEM, glutaraldehyde-fixed specimens were treated with 2% osmium tetroxide buffered in 0.1 mM cacodylate buffer. Specimens were dehydrated with graded ethanol to 2 × 100% ethanol and 2 × propylene oxide (EM Sciences). The samples were embedded in pure Epon resin. One micrometer tissue sections were cut, stained with toluidine blue, and examined at the light microscopic level. Ultrathin sections were cut from areas of interest, stained with lead citrate and uranyl acetate (both from EM Sciences), and observed in a Philips 400 Transmission Electron Microscope. (Philips Instruments, Eindhaven, The Netherlands).

RESULTS

Biochemical analysis of collagen fragmentation in aged and photodamaged skin In the first series of experiments, skin biopsies were obtained from the forearm of nine individuals with severe photodamage. Sun-protected hip skin from each individual served as a control. The skin samples were assessed for the presence of collagen fragments as described in the *Materials and Methods* section. There were significantly more collagen fragments (as a percentage of total collagen) in the skin samples from the severely photodamaged forearm skin than in matched samples from hip skin (**Fig 1*A***). In a like manner, when sun-protected hip skin samples were obtained from eight individuals aged 18–29 y and from eight individuals 80 y or older, the presence of collagen fragments was increased in the old skin relative to the young (**Fig 1*B***). Finally, sun-protected skin samples from young and old individuals were assessed for total collagen content as a function of total protein. **Figure 1(*C*)** demonstrates that the collagen content (per mg of total protein) was approximately 30% lower in skin samples from old individuals than in corresponding samples from young individuals.

Ultrastructural features of collagen in aged and photodamaged skin Next, we examined sun-protected hip skin and severely photodamaged forearm skin from five different individuals by SEM and TEM. **Figure 2** demonstrates ultrastructural features of sun-protected hip skin from one representative member of the cohort. By SEM, sun-protected skin demonstrated a uniform lattice of collagen fibrils, distributed within the dermal layer (**Fig 2*A,B***). Small bundles of collagen fibrils could also be seen in sections from the same skin samples (**Fig 2*B***, *arrow*). **Figure 2(*C*)** demonstrates the appearance of the collagen fibrils by TEM. Individual collagen fibrils as well as dense collagen bundles can be seen. Periodicity

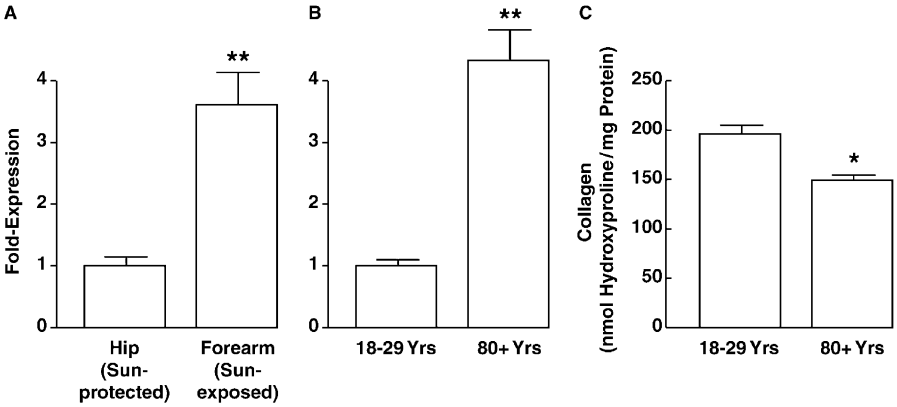

Figure 1. (*A,B*) Collagen fragmentation in photoaging and natural aging. Values shown represent the amount of hydroxyproline released from skin extracts by exposure to α-chymotrypsin. Values shown for sun-protected (hip) skin in (*A*) and for skin from young individuals (18–29 y) in (*B*) have been set at 1.0 and the values for photoaged skin and skin from old individuals (80 y or older) expressed relative to this. Statistical significance was determined using the Student's t test ($n = 9$ and $n = 8$, respectively, $^{**}p < 0.01$). (*C*) Total collagen (soluble and insoluble hydroxyproline) in sun-protected skin from young and old individuals. Statistical significance was determined using the Student's t test ($n = 6$, $^{*}p < 0.05$).

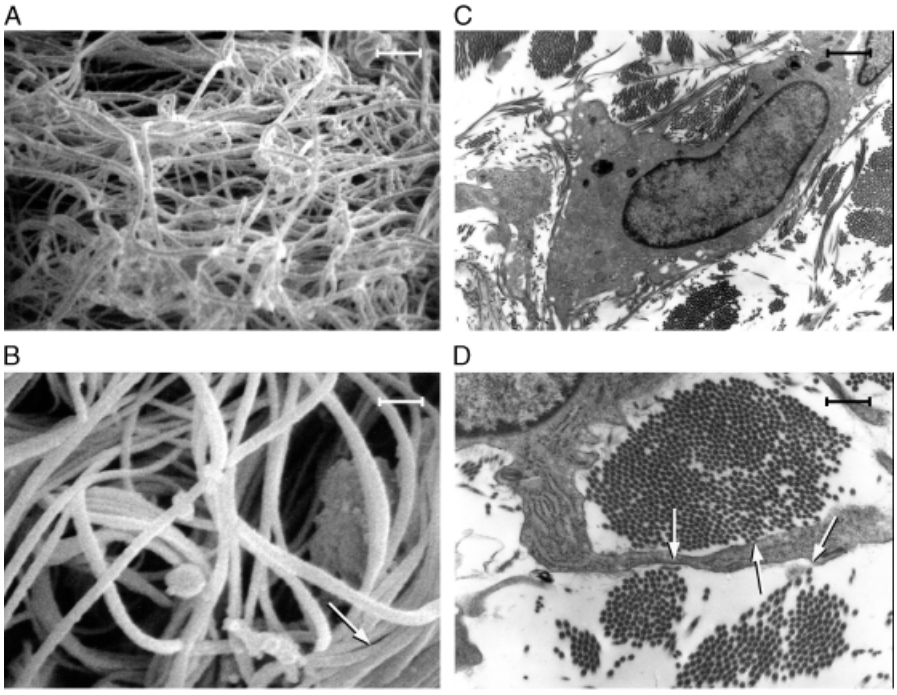

Figure 2. Ultrastructural appearance of sun-protected hip skin. (*A,B*) SEM pictures demonstrating uniform distribution of intact collagen fibrils and small collagen fibril bundles (*arrow*). (*C,D*) TEM pictures. (*C*) Demonstrates a fibroblast surrounded by and in contact with intact collagen fibrils and bundles of collagen fibrils in both longitudinal and transverse orientation. At higher magnification (*D*), a fibroblast cell process can be seen (*arrows*). Cell processes are frequent sites of contact with intact collagen. *Scale bars*: (*A*) 1.33 μm; (*B*) 0.67 μm; (*C*) 3.57 μm; (*D*) 1.25 μm.

of individual fibrils is apparent. Morphometric analysis of the transmission electron micrographs demonstrated significant uniformity in fibril width and periodicity. Consistent with established values (Bevelander and Ramaley, 1974), the average width of the individual fibrils was $784 + 76$ angstroms and the periodicity was $470 + 35$ angstroms. It was impossible to determine an average fibril length as many of the fibrils were not fully in the plane of the photograph. Fibrils that appeared to be entirely within the plane of the picture ran for 8 μm or more. **Figure 2(*C,D*)** also demonstrate the close apposition of an interstitial fibroblast to individual collagen fibrils and collagen fibril bundles. A majority of the cells were characterized by the presence of extensive cell processes, and these processes were always points of contact between the cell and the collagen matrix (**Fig 2*D***, *arrows*). Within cells, large amounts of endoplasmic reticulum (indicative of protein synthesis) could be seen (**Fig 2*D***).

Ultrastructural features of severely photodamaged forearm skin are shown in **Fig 3**. In the photodamaged skin samples, breaks in the collagen fibrils were apparent. In places, the broken fibrils were twisted and clumped. There were large areas of the dermis devoid of identifiable collagen, whereas in other areas, dense aggregates of twisted, clumped collagen were seen. These features are demonstrated at the SEM level in **Fig 3(*A,B*)**. **Figure 3(*C,D*)** demonstrate the appearance of severely photodamaged skin at the TEM level. Degradation of individual collagen fibrils (resulting in fiber shortening, in addition to a loss of fine structure and periodicity) can be seen along with the presence of unidentifiable amorphous (elastotic?) material. Interaction of interstitial fibroblasts with damaged collagen fibrils (**Fig 3*C***) and with acellular debris (**Fig 3*D***) can be seen. The features noted in **Figs 2 and 3** were consistent among the five individuals with extensive photodamage examined in this way.

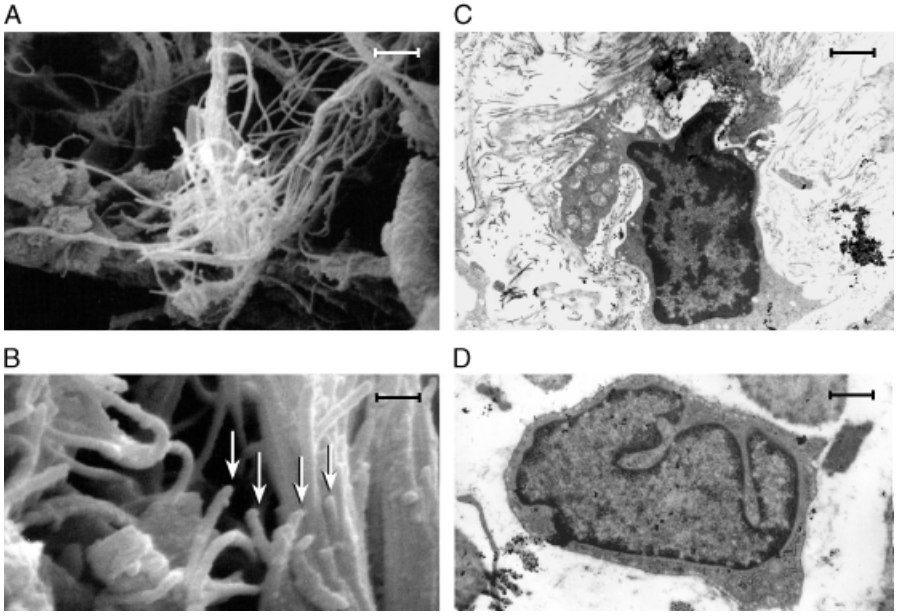

Figure 3. Ultrastructural appearance of photodamaged forearm skin. (*A,B*) SEM pictures demonstrating broken collagen fibrils. At higher magnification (*B*), numerous breaks in the fibers are apparent. (*C,D*) TEM pictures demonstrating a lack of intact collagen fibrils, the presence of disrupted matrix and a large amount of amorphous material. Cells in contact with a mixture of intact and fragmented collagen fibers (*C*) and with amorphous material (*D*) are apparent. *Scale bars*: (*A*) 1.33 μm; (*B*) 0.67 μm; (*C*) 3.57 μm; (*D*) 2.17 μm.

In addition to examining sun-protected hip skin from individuals with extensive (forearm) photodamage, we also examined sun-protected skin from three individuals between the ages of 18 and 29 y. Three individuals 80 y or older were examined in parallel. Skin from 18 to 29 y old individuals was not significantly different at the ultrastructural level from sun-protected skin of a majority of the individuals with extensive photodamage (average age 62 y). In contrast, focal areas of fragmented collagen were observed in sun-protected hip skin from individuals aged 80 y or older. Although such areas were less pervasive in the sun-protected skin from these individuals than in severely photodamaged skin, where fragmented collagen was observed, it was qualitatively indistinguishable from the collagen damage noted in photodamaged skin (not shown).

Fragmentation of collagen *in vitro*: biochemical and ultrastructural features **Figure 4** demonstrates fragmentation of three-dimensional lattices of polymerized type I collagen *in vitro* by MMP-1. In the presence of buffer alone (**Fig 4**, lane 1), α1 and α2 chains of type I collagen were visible. The α1 and α2 chains were extremely faint, however, due to the fact that most of the intact collagen remained polymerized in the lattices. No collagen fragments were detected. In the supernatant fluid from lattices exposed to 400 ng of MMP-1, one-fourth and three-fourths size fragments were present (**Fig 4**, lane 2). In order to estimate how much collagen was released from the lattices during the 5 h incubation, a stock solution of collagen was completely digested by exposure to 1 μg of MMP-1 for 18 h. A dose–response curve was constructed and the amount of one-fourth and three-fourths size fragments in the 5 h digestion determined from this. Based on this, we estimate that 30–40% of the type I collagen molecules in the lattice were converted into fragments. Measurable fragmentation also occurred with 200 or 100 ng of enzyme, but it was significantly reduced as compared with 400 ng of enzyme. No detectable fragmentation was observed after exposure to lower enzyme concentrations (50 ng) (not shown). At the macroscopic level, the three-dimensional structure appeared unchanged after exposure to 400 ng of enzyme; however, upon addition of 8×10^4 dermal fibroblasts to the MMP-1-treated collagen, contraction of the collagen occurred over a 2 d period, whereas no contraction was seen in control collagen lattices. The ability of dermal fibroblasts to contract collagen after exposure to MMP-1 under conditions in which intact collagen is resistant to contraction is consistent with what has been described in recent reports (Varani *et al*, 2001, 2002).

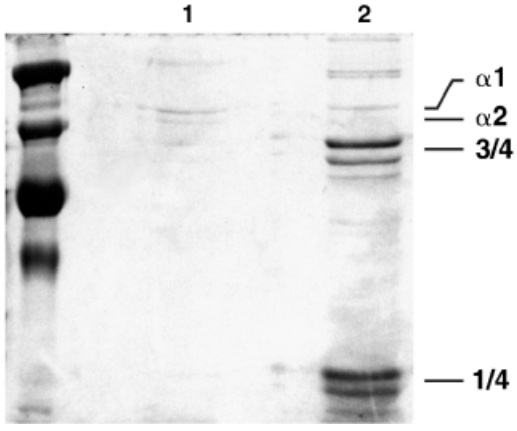

Figure 4. Cleavage of intact type I collagen by MMP-1. *Lane 1*: buffer alone. Intact α1 and α2 collagen chains are barely detectable and there is essentially no fragmentation. *Lane 2*: MMP-1. Intact α1 and α2 collagen chains are barely detectable, but three-fourths and one-fourth size fragments are prominent.

Control collagen lattices and lattices exposed for 5 h to MMP-1 (400 ng per 0.5 mg of collagen) were examined by SEM and TEM. By SEM, control lattices revealed intact, regularly distributed collagen fibrils that were very similar in appearance to the collagen fibers observed in sun-protected skin biopsies (**Fig 5*A***). Small bundles of collagen fibrils were also observed, but they were relatively sparse. Individual collagen fibrils and small fibril bundles were also apparent at the TEM level (**Fig 5*B***). Average width of the polymerized collagen fibrils (793 ± 70 angstroms) was almost identical to that of naturally occurring collagen. Fibril periodicity was not readily apparent in most individual fibrils. When periodicity was seen, it was similar

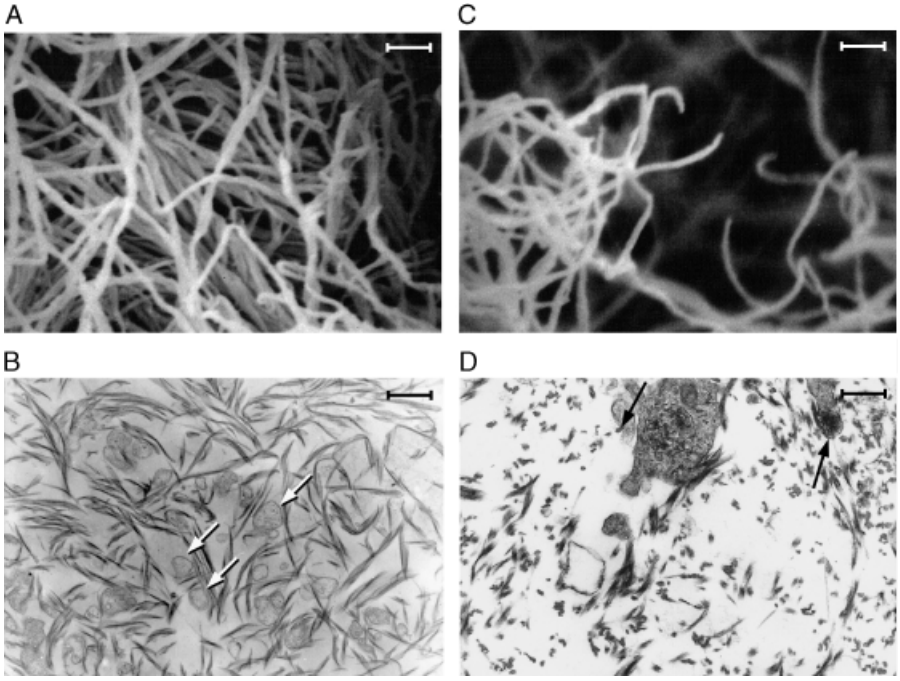

Figure 5. Ultrastructural appearance of control three-dimensional collagen lattices and collagen lattices after exposure to MMP-1. (*A,B*) SEM and TEM pictures of intact collagen lattices. At the SEM level (*A*), a network of intact collagen fibrils is apparent. At the TEM level (*B*), individual collagen fibrils can be seen. Numerous fibroblast cell processes can be seen, and these are points of contact with the collagen fibers (*arrows*). (*C,D*) SEM and TEM photographs of collagen lattices following exposure to MMP-1. At the SEM level (*C*), breaks in the collagen fibers and collagen clumping are seen. By TEM (*D*), few intact collagen fibrils are visible, and cell processes are in contact with shortened fibrils and debris (*arrows*). *Scale bars*: (*A*) 1.0 μm; (*B*) 2.17 μm; (*C*) 1.0 μm; (*D*) 2.17 μm.

to that of intact collagen *in vivo* (average of 490 angstroms). Periodicity of the collagen fibrils reflects precision in the alignment of the α1 and α2 chains of the intact collagen molecules. The precise alignment is maintained by extensive cross-linking. As there is no cross-linking in the reconstituted collagen lattices, there is more freedom of movement of the collagen molecules. Ultimately, the alignment of the α1 and α2 chains in the reconstituted collagen fibrils is less precise. The average length of the fibrils in the collagen lattices (1.2 ± 0.2 μm) was shorter than the average length of the collagen fibrils *in vivo*; however, whether this reflects an absolute difference in average fibril length or simply indicates that the fibrils were more tangled and therefore out of the plane of the photograph cannot be determined. The major difference between the collagen lattices and collagen in intact skin was in concentration. Routinely, the collagen concentration in the lattices was 1 mg per ml (concentrations as high as 4 mg per ml have been used with similar results). In contrast, skin collagen concentration may be as high as 70 mg per ml, although density varies greatly between the papillary and reticular zones. Where cells were seen in the collagen lattices, numerous cell processes in contact with the collagen fibrils were evident (**Fig 5*B***, *arrows*).

When MMP-1-degraded collagen lattices were examined by SEM (**Fig 5*C***), breaks in individual collagen fibrils were evident, and in places, aggregates of the fragmented collagen fibrils could be seen. Other areas within the lattices were devoid of collagen altogether. Examination of one of these areas by TEM revealed prominent collagen fragmentation with loss of fine structure (**Fig 5*D***).

DISCUSSION

Repeated exposure to ultraviolet irradiation from the sun over years or decades upregulates MMP (Fisher *et al*, 1997) and produces the clinically appreciable matrix destruction characteristic of photodamage. The consequences of damage to the collagen matrix is compounded by the fact that in the severely damaged matrix, new collagen synthesis is reduced (Griffiths *et al*, 1993; Talwar *et al*, 1995). The relationship between collagen damage and decreased collagen synthesis is not fully understood. In recent studies, it was demonstrated that when three-dimensional lattices of polymerized type I collagen were partially degraded by exposure to organ culture fluid from human skin (containing a mixture of active MMP) or by exposure to MMP-1 alone, production of type I procollagen by fibroblasts grown on the partially degraded lattices was decreased (Varani *et al*, 2001, 2002). On the basis of these observations, it was suggested that collagen fragmented by MMP *in vivo* might contribute to the loss of collagen synthetic activity that characterizes photodamaged skin.

In this study we have used a combination of biochemical and ultrastructural approaches to compare photodamaged skin *in vivo* and damage to collagen lattices exposed to MMP-1 *in vitro* and to examine fibroblast interaction with the damaged matrix in both situations. The results indicate that in both severely photodamaged skin *in vivo* and collagen lattices exposed to MMP-1 *in vitro*, there were numerous breaks in the collagen fibrils, clumping of fragmented collagen and an irregular (nonuniform) distribution of the collagen. As a result, some areas (both *in vivo* and *in vitro*) were devoid of collagen fibers, whereas others contained dense clumps of collagenous material. Fibroblasts in close apposition to degraded collagen fibrils were evident. These ultrastructural similarities between degraded collagen in severely photodamaged skin and MMP-1-degraded collagen *in vitro* suggest that MMP-1-mediated damage to collagen *in vitro* may provide insight into mechanisms of collagen loss in intact skin. Based on these results we hypothesize that damaged collagen *in vivo* leads to loss of mechanical tension on resident fibroblasts and concomitantly to reduced procollagen synthesis in a similar manner to what has been described *in vitro* (Varani *et al*, 2001, 2002).

Although the initial focus of this work was on collagen destruction in severely photodamaged skin, the results may also be applicable to skin damage during the natural aging process.

Similar abnormalities to those seen in photodamaged skin were also observed (though not as extensively) in sun-protected skin in chronologic aging, i.e., in skin from individuals aged 80 y or older. There are, however, additional abnormalities in photodamaged skin (i.e., the accumulation of elastotic material and other acellular debris) that are not prominently seen in aged, sun-protected skin (Smith *et al*, 1962; Maloney *et al*, 1992; Marks, 1992; Bailey, 2001). These same features of photodamaged skin are also, of course, not present in the *in vitro* collagen lattices. As fibroblasts interact with the amorphous material as well as with identifiable fragments of collagen (see **Fig 3**), these interactions could further modulate collagen synthesis in ways not appreciated *in vitro*.

An intriguing question is why fragmented collagen molecules are not broken down into smaller pieces and eliminated—either *in vivo* or in collagen lattice cultures exposed to MMP from skin. Collagen is known to be unstable at physiologic pH (Leikina *et al*, 2002) and the enzymes that further degrade the high molecular weight fragments produced by MMP-1 (especially MMP-9; 92 kDa gelatinase B) are present in the skin and upregulated by ultraviolet light (Fisher *et al*, 1996). Whereas the answer is not known with certainty, our recent studies provide insight. In these studies we showed that when MMP-9 was added to collagen lattices along with MMP-1, the three-fourths and one-fourth fragments generated by MMP-1 were further degraded and cleared (Varani *et al*, 2002). The apparent failure of MMP-9 to function effectively *in vivo* may suggest that it is unavailable—either because there is too little MMP-9 relative to the amount of MMP-1 present or because it is tied up with MMP inhibitors. With regard to stochiometry, each $\alpha 1$ or $\alpha 2$ chain of type I collagen provides a single molecule of substrate for MMP-1. In contrast, each three-fourths and one-fourth fragment produced by MMP-1 presents multiple sites for cleavage by MMP-9. Thus, the effective rate of bond cleavage would need to be much higher with MMP-9 than MMP-1 for the gelatinolytic enzyme to keep up with the collagenase. Alternatively, the level of MMP-9 activity may be lower than it appears to be because of inhibitors present in the skin. Whereas tissue inhibitors of metalloproteinases are not completely selective among MMP, there is a degree of specificity. In particular, tissue inhibitor of metalloproteinase-1 has a high affinity for MMP-9 (Howard and Banda, 1991; Goldberg *et al*, 1992; Bodden *et al*, 1994). We have already demonstrated that virtually all of the MMP inhibitor activity detected in human skin is due to tissue inhibitor of metalloproteinase-1 (Chi *et al*, 1998). Thus, it may be that the available MMP inhibitor is bound up with MMP-9 while leaving most of the MMP-1 free. Additional studies will be needed to distinguish between these two possibilities, which are, in any case, not mutually exclusive.

Based on these results and on our recent studies, we suggest that the presence of fragmented collagen in aged/photodamaged skin actively contributes to the loss of procollagen synthesis. How damaged collagen brings about a reduction in new collagen synthesis is not fully understood. *In vitro* studies have documented the relationship between fibroblast-induced collagen contraction, loss of mechanical tension on the cells, and decreased collagen synthetic activity (Clark *et al*, 1995). The loss of mechanical tension could lead to reduced collagen production by preventing effective receptor–ligand interactions and/or by interfering with intracellular signaling events (Lin and Grinnell, 1993; Boudreau and Jones, 1999; Aplin *et al*, 2001) that are necessary for collagen synthesis. The innate sensitivity of fragmented collagen to contraction (Varani *et al*, 2001, 2002) could therefore underlie reduced procollagen synthesis in the presence of the damaged matrix. Another possibility is that reduced procollagen elaboration by fibroblasts on fragmented collagen reflects increased breakdown rather than decreased synthesis. Past studies have demonstrated a correlation between increased MMP elaboration and reduced collagen synthesis in fibroblasts (Lovell *et al*, 1987; Millis *et al*, 1989).

In conclusion, the findings presented here show that exposure of three-dimensional collagen lattices to MMP-1 *in vitro* produces fragmentation of the collagen fibrils and clumping of the fragmented collagen. Collagen fragmentation and clumping are also observed in the dermis of aged/photodamaged skin *in vivo*. These findings are consistent with the suggestion that MMP-mediated collagen damage could be responsible, at least in part, for the reduction in collagen production seen *in vivo* in aged/photodamaged skin. These findings support the use of three-dimensional collagen lattice cultures as an *in vitro* model for understanding mechanisms by which damaged collagen influences biologic function.

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